immunological subject matter related thereto. Applicants address certain issues respecting the interview below.

1. With respect to the amendment to the specification, Applicant requests that the word "protein" be deleted from all instances where the word is used in relation to the word "GM-1". Applicants respectfully bring to the Examiner attention that GM-1 is a ganglioside that is also a pentasaccharide, i.e., a complex sugar, and is not a protein *per se*. Applicants have attached hereto page 553 from Stryer's Biochemistry, third edition, Freeman editors, which shows that any skilled artisan knows this is an improper use of "protein" and that GM-1 is not a protein but in fact a carbohydrate containing ganglioside. Reference to "GM-1 protein" is therefore essentially improper context, analogous to referring to a zucchini or some other vegetable as a fruit. Importantly, GM-1 is properly referred to in the specification as a ganglioside and pentasaccharide, such as on pages 42, line 7, and page 104, line 7-8, 21, 22, and 28. Additional description is found at page 104 lines 23-27. Therefore, deletion of "protein" in context with GM-1 is not new matter with respect to the application. In two locations to make the context of the sentence continue to read intelligently, the word "molecule" must be inserted in place of "protein. These locations are at page 104, line 8, and page 106, line 6, respectively. Additionally, on page 93, line 11, "protein" must be deleted and replaced with "molecule" as well. Here, use of "protein" indirectly is referencing GM-1.

Applicant further notes a need for three other corrections in the specification. Specifically, at page 94, line 4, the word cholera is misspelled. At page 94, line 13, the word "(source)" must be replaced with the name of the company from which the referenced monoclonal was obtained. Addition of the company name should not comprise new matter as such materials are commonly available and in any event one skilled in the art would know where to obtain such material. Finally, at page 105, line 7, the word "involve" should be past tense.

2. With respect to the claims, as discussed at the interview, Applicant traverses the grouping of the claims as delineated by the Examiner in the restriction requirement mailed January 5, 2001, and requests that the grouping be revised to more accurately reflect the different inventions claimed in this application. Specifically, among the various inventions claimed in the application, the Examiner has grouped the claims into thirteen different groups, and among such groups has restricted claims drawn to artificial APCs into 6 individual groups (i.e., Groups I-

VI), collectively, claims 1-149. Moreover, claims drawn to a method of making artificial APCs (claims 150-161) have been grouped in to a separate Group VII.

Applicant respectfully traverses the restriction respecting the claims drawn to artificial APCs, Groups I-VI, and request that the restriction be withdrawn. Specifically, the Examiner states that the inventions I-VI are unrelated. However, this is absolutely not the case. A careful review of the claim sets of each group will reveal that Applicant has merely organized the claims to the artificial APC invention into six claim sets from the broadest to the most restricted independent claims. Claims dependent respectively from each set are arranged in increasing addition and organization of the APC subcomponents. Essentially, the whole of claims 1-149 are directed to not seven different types of structure to be used differently, but rather to the same invention having varying breadths of claim scope. Importantly, the claims of each succeeding group contain the same limitations of the previous group with the minor exception that the independent claim of group VI contains an additional element of a solid support. However, reference to a solid support is also found in the dependent claims 25, 52, 79, 107, and 120 of each of the Groups I-V. Thus, Groups I-VI should be viewed as drawn to the same invention.

As the Examiner is well aware, it is a long standing policy of the PTO to allow diverse and broadly structured claims, and Applicant is merely taking advantage of his right to structure claims in such manner. Moreover, each of these groups are recognized by the Examiner as having both the same Class and subclass with regard to classifications used in searching the subject matter. Given that the claims are identically classed, there should be no additional or extensive burden place on the Examiner to conduct a collective search for the claims grouped as one related set. Thus, Applicant respectfully requests that claims 1-149 be kept together for prosecution in this application.

Additionally, Applicant requests that claims 150-161 be considered in this application because the process of making the claimed APC is unique and cannot be said to make other materially different products nor can they be made by other and materially different processes.

In order to aid the efficient and timely prosecution of such claims and to provide additional support for keeping the prosecution of claims of Groups I-VII in the same application, Applicant has amended claims 1-161. For simplicity, due to the complexity of the claims, their large number, and to make review of claims easier, given the new burdens placed on presentation of claim amendments by the new rules imposed by the PTO earlier this year, Applicant has

cancelled claims 1-161 and replaced them with claims 220-380. Specifically, the new claims 220-367 are provided as a complete set paralleling the content of original claims 1-149 of Groups I-VI. Claims 368-380 parallel original claims 150-161 of Group VII.

To each of the independent claims 220, 246, 273, 300, 328, and 342 (which parallel original independent claims 1, 27, 54, 81, 109, and 124, respectively) is included an element drawn to a "molecule for orienting a molecule of interest". Applicant believes this element is fully supported by the description in the specification relating to the use of GM-1 and cholera toxin β subunit in the claimed liposomes for orienting the MHC, accessory, co-stimulatory, cell modulation, adhesion, and irrelevant molecules discussed therein. Extensive discussion can be found throughout the specification concerning use of GM-1 and cholera toxin β subunit for such orientation. For example, reference may be found at page 12, lines 18 to page 13, line 8. In this excerpt, the specification states on page 13, line 3, "All of these molecules are incorporated into the liposomes of the artificial APC in a free floating format", referencing the fact that, as stated on page 12 that the protein of interest may be connected to the cholera toxin subunit which is part of the anchoring mechanism comprising GM-1 and the β subunit which as a unit is "free floating" in the lipid membrane.

Additional similar references include page 13, lines 20-23, which discusses "proper orientation". Page 14, lines 26 to page 15, line 16 discusses proper orientation and here it is specifically referred to as "**facing outward**". Page 18, lines 4-6 also discuss orientation. page 19, lines 10-15 note that the MHC:antigen complexes float freely. Page 20, lines 9-10 note proper orientation. Page 22, lines 3-19 discuss the binding of the molecules of interest to cholera which binds to GM-1 and use of phospholipids and cholesterol for free migration of molecules of interest around the lipid layer. At page 23 is found a basic discussion of APC formation. Also at page 19, line 19, is discussed that "each of these molecules incorporated may be prelinked to cholera toxin.

Still additional references to proper orientation may be found at page 40, lines 17 to page 41, line 8, and page 41 lines 24 to page 42 line 9, wherein it is stated (page 41 lines 27-28) that GM-1 and subunits of cholera toxin may be attached to any of the aforementioned molecules. Page 43, lines 3-10 discuss proper orientation such that 90% proper orientation can be achieved. See also page 44, lines 25-26, page 45, line 16-17, and page 46, lines 25-26.

Further, page 47, lines 12-20, provide a discussion noting that non-lipid molecules (e.g. MHC:antigen complexes, GM-1 bound to cholera) freely migrate in the lipid bilayer and provide proper orientation of molecules of interest. Further references are found at pages 49, lines 28-30, and page 104, lines 5-7.

Applicants point out further support in the specification to references to "raft" structures as discussed on page 94, lines 4 and 8, and page 106, line 7. Applicant respectfully points out that the raft structure is used in the same context as having free mobility in the lipid membrane in the form of a raft comprising GM-1 and cholera toxin β subunit. Such free mobility (allowed by such raft structure) is referred to throughout the specification such as found on pages 11, 19, 22, 42, 45, 47, and 93 wherein are discussed free floating and/or free migration of the raft components. Such composition is not only unique in the present context but also provides utterly unobvious and surprising results with respect to the response by T cells that come into contact with the artificial APCs of the invention.

As is readily apparent, Applicant has substantially made reference in the specification to use of GM-1 and cholera β subunit for use in orientation of the molecules of interest which are the MHC, accessory, co-stimulatory, cell modulation, adhesion, and irrelevant molecules. Thus, there is ample support under 35 U.S.C. § 112 for written description and enablement so as to allow inclusion in the new independent claims 220, 246, 273, 300, 328, 342, and 368 an element drawn to "molecules for orienting molecules of interest." Additionally, there need not be any transitional language to delineate "molecules for orienting" from "molecules of interest" because the claims as drafted are clear as to which "molecules" are being referred to for each instance in which these terms are used.

Applicant respectfully brings to the Examiner's attention that newly added independent method of making claim 368 also includes the "molecules for orienting molecules of interest" element used in the composition claims 220-367. Importantly, the method of making claims are closely linked to the composition claims through the novel use of the molecules for orienting molecules of interest wherein GM-1 and cholera toxin are part of these method of making claims. Since each of the independent claims 220, 246, 273, 300, 328, and 342 includes such element, the claimed compositions of matter and method of making claim 368, are even more closely related and unique in composition and making than indicated as originally drafted.

Given the inclusion of the element "molecules for orienting molecules of interest" (i.e., raft elements) into the main body of the independent claims, Applicant wishes to elect new claims 220-380 drawn to a common invention. Given the content of the newly drafted and elected claims, Applicant believes that such claims to artificial APCs as claimed in new claims 220-367 and method of making the artificial APC claims 368-380 are now in condition for immediate allowance.

3. Please charge our deposit account (50 1273) in the amount of $890.00 for a three month extension to and including July 5, 2001.

4. Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

Given the above amendments and remarks, Applicants believe the application is now in condition for allowance. No fee other than the fee required for extension of time is believe due respecting the instant response, however, if any fee is due please charge our deposit account Number 50/1273 in the appropriate amount. If the If the Examiner needs to reach me, my direct telephone number is (858) 720-2757.

Respectfully submitted,
Brobeck, Phleger & Harrison LLP

Dated: 8/8/01

By: _____
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The word "protein" is deleted at designated locations throughout the specification so that the sentences wherein such word is found read without the specified word "protein". Additionally, the amendments include minor typographical errors and replacement with correct words in certain instances as follows:

1. Page 12, sentence beginning line 24 to 27 is amended:

Moreover, the current invention further provides for proper orientation of each of these molecules within the artificial APC membrane by a novel use of an anchoring mechanism comprising GM-1 ~~protein~~ and the β subunit of cholera toxin.

2. Page 12, sentence beginning line 29 to page 13, line 1 is amended:

By attaching the cholera toxin subunit to the molecule of interest, the cholera toxin may be bound by the GM-1 ~~protein~~ that is incorporated into and has affinity for the nonpolar region of the artificial APC membrane.

3. Page 17, sentence beginning line 11 to 14 is amended:

There is no relation inherent or otherwise to the current invention, nor is there insight disclosed as to liposome construction containing co-stimulatory and adhesion molecules or protein orientation mechanisms such as the binding of cholera toxin by GM-1 ~~protein~~, or fused or linked moieties to the MHC, functional or accessory proteins of interest.

4. Page 22, sentence beginning line 9 to 11:

In a preferred embodiment, the cholera toxin moiety remains in the APC's interior by binding to GM-1 ~~protein~~ that is incorporated into the APC's lipid interior.

5. Page 22, sentence beginning line 29 to page 23, line 3, amended :

In still another embodiment, the APC comprises labels wherein a label is associated with at least one of the group selected from the group consisting of a lipid bilayer of the liposome

components, a lipid of the liposome, an antigen, an MHC molecule, a co-stimulatory molecule, an adhesion molecule, a cell modulation molecule, GM-1 ~~protein~~, cholera toxin β subunit, an irrelevant molecule, and an accessory molecule.

6. Page 40, paragraph beginning line 17 to page 41, line 8 is amended:

Fig. **27A-C** is a schematic showing methodology for orienting molecules of interest in the APC liposome matrix. In **27A**, a molecule of interest such as MHC, functional, accessory, adhesion, or irrelevant molcule, may be synthesized by recombinant methods well known to those skilled in the art and linked to GM-1 ~~protein~~ by a linker and properly oriented in the APC membrane. In **27B**, a molecule of interest may be constructed as a fusion protein with cholera toxin β subunit and the fusion protein anchored in proper orientation in the APC membrane by the cholera toxin moiety binding to GM-1 ~~protein~~. In **27C**, a cholera toxin subunit may be chemically linked to SPDP linker (Pierce) and then attached to a molecule of interest followed by anchoring to a GM-1 containing APC. In any of the above, the cholera toxin, GM-1 ~~protein~~, linkers may be synthetically produced. In the figure, A represents a gene for a molecule of interest, B represents the gene for the β subunit of cholera toxin, A1 is an expression vector, A2 represents expression and isolation of the cloned gene, A3 is an expressed molecule of interest, B1 is a fusion protein of a molecule of interest and cholera toxin, A4 is a linker, A5 is an artificial APC containing GM-1 ~~protein~~, A7 is a partial view of an artificial APC wherein the molecule of interest is directly linked to the GM-1 ~~protein~~, C is choler toxin subunit, C1 is a linker, C2 is a molecule of interest, C3 is a choler toxin subunit attached to a linker, C4 is a molecule of interest linked to a choler toxin subunit, E represents a liposome bilayer, E1 shows GM-1 ~~proteins~~, E2 is an artificial APC containing GM-1 ~~protein~~, and F is a partial view of an artificial APC having a molecule of interest bound to the APC by the binding interaction of the GM-1 ~~protein~~ and cholera moiety.

7. Page 42, two sentences beginning line 6 to line 9 are amended:

Other lipid membrane components include GM-1 ~~protein~~ which is a transmembrane pentasaccharide ~~protein~~ and associates in part with nonpolar regions of the liposome matrix. This GM-1 ~~protein~~ can be used in association with cholera toxin β subunit to orient molecules of interest in the liposome matrix.

8. Page 43, sentence beginning line 3 to line 8 is amended:

With respect to the incorporation of each of the aforementioned MHC, accessory, co-stimulatory, adhesion, modulation, and irrelevant molecules in the artificial APC, proper orientation of these molecule's active centers may be provided by combining the molecules with the β subunit of cholera toxin so that the cholera toxin subunit can be recognized and bound by GM-1 ~~protein~~ which is incorporated into the liposome membrane matrix.

9. Page 44, sentence beginning line 5 to line 10 is amended:

In still another embodiment, the APC comprises labels wherein a label is associated with at least one of the group selected from the group consisting of a lipid bilayer of the liposome components, a lipid of the liposome, an antigen, an MHC molecule, a co-stimulatory molecule, an adhesion molecule, a cell modulation molecule, GM-1 ~~protein~~, cholera toxin β subunit, an irrelevant molecule, and an accessory molecule.

10. Page 44, sentence beginning line 23 to line 28 is amended:

In another embodiment, any of the molecules of interest (MHC, accessory, co-stimulatory, adhesion, modulation, irrelevant molecules) can be bound to the β subunit of cholera toxin and GM-1 ~~protein~~ can be included in the APC lipid matrix to provide a means for proper orientation of the molecules of interest such that their active centers are oriented to facilitate interaction with T cells and other components external to the APC.

11. Page 47, sentence beginning line 17 to 20 is amended:

Moreover, the lipid bilayer may also include accessory molecules such as cholesterol to provide elasticity in the bilayer and GM-1 ~~protein~~ to provide an anchor for orientation of cholera β subunit comprising molecules of interest.

12. Page 49, sentence beginning line 28 to 30 is amended:

Further, GM-1 ~~protein~~ is incorporated into the lipid layer matrix providing a means by which the cholera toxin portion can be bound and the molecule of interest properly oriented in the lipid layer.

13. Page 93, the sentence beginning line 9 and ending line 12 is amended:

To visualize free movement of the TCR in the T cell membrane, we employed a system where FITC-conjugated cholera toxin, a molecule known to combine with the intracellular portion of transmembrane ~~proteins~~ **molecules**, is introduced into the T cells.

14. Page 94, the sentence beginning line 4 is amended:

In Fig. 16A-D, we visualized comigration of the ~~colera~~ **cholera** raft and the TCR, by incubating T cells with PE-conjugated monoclonal antibody (Pharmigen).

15. Page 94, the sentence beginning line 12-14 is amended:

In experiments described in Fig. 18A-D, we visualized the TCR using an Alexa red-conjugated anti CD3 monoclonal ~~(source)~~ **(Molecular Probes, Eugene OR)** and the liposomes using FITC-conjugated streptavidin which bound to the biotin at the N-terminal of the OVA peptide.

16. Page 104, two sentences beginning line 7 to line 11 are amended:

This mechanism uses GM-1 pentasaccharide ~~protein~~ which is a transmembrane ~~protein~~ **molecule** and has an affinity for binding the β subunit for cholera toxin. When the GM-1 ~~protein~~ is associated with the liposome membrane of the APC, it can be used to bind cholera toxin which in turn can be attached to the molecule of interest.

17. Page 105, sentence beginning line 7-8 is amended:

Since the β subunit is primarily ~~involve~~ **involved** in the binding to the GM1 pentasaccharide, the α subunits are not necessary.

18. Page 106, sentence beginning line 6 to line 7 is amended:

The GM1 ~~protein~~ is a transmembrane ~~protein~~ **molecule** which can be associated with the "raft" or freely mobile molecules of interest in the lipid membrane.

19. Page 106, sentence beginning line 12 to line 13 is amended:

The recombinant product can be purified and linked to GM-1 ~~protein~~ that is in an artificial APC.

20. Page 106, two sentences beginning line 15 to line 21 are amended:

The fusion product can be purified and mixed with an artificial APC containing GM-1 ~~protein~~ where the cholera moiety will bind to the GM-1 ~~protein~~. Additionally, as shown in Fig. **27C**, the cholera toxin (whether natural or recombinant) can be attached to a linker, such as N-succinimidyl [3-(2-pyridyl) dithio] propionate, either to a complete β subunit molecule or during synthesis of a recombinant toxin molecule, the product of which can be then mixed with a GM-1 ~~protein~~ containing artficial APC.

21. Page 106, sentence beginning line 24 to line 26 is amended:

Once the GM1 ~~protein~~ is incorporated into the liposome of the APC, cholera toxin-cojugated surface proteins can then be cross-linked.

In the claims:

1. Please cancel claims 1-161 without prejudice to their future prosecution.

2. Please add new claims 220-380 as delineated above at pages 3-29 of this Preliminary Amendment.